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Brassinosteroids on the oxidizing and hydrolyzing enzymes of radish plants – A study

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Abstract
The effect of 24-epibrassinolide and 28-homobrassinolide on the activities of two oxidizing enzymes (catalase and peroxidase) and two hydrolyzing enzymes (ribonuclease and protease) of radish plants were studied. Both the brassinosteroids stimulated the activity of the oxidizing enzyme, catalase of the radish plants. The activity of the other oxidizing enzyme peroxidase was decreased by the application of 24-epibrassinolide and 28-homobrassinolide. Brassinosteroid-treatment resulted in lowered protease as well as ribonuclease activity.

Keywords: 24-epibrassinolide, catalase, 28-homobrassinolide, peroxidase, protease, ribonucleasae.

INTRODUCTION

Radish (*Raphanus sativus*) is an edible root vegetable belonging to the family *Brassicaceae* which is grown through the world. It is a well established fact from time immemorial that plants are the critical components of dietary food chains in which they provide almost all the essential mineral and organic nutrients to humans.

Brassinosteroids are a new type of polyhydroxy steroidal phytohormones with significant growth promoting influence [1, 2]. Brassinosteroids (BRs) were discovered in 1970 by Mitchell and his co-workers [3] and were later extracted from the pollen of *Brassica napus* L [4]. BRs are considered ubiquitous in plant kingdom as they are found in almost all the phyla of the plant kingdom like alga, pteridophyte, gymnosperms, dicots and monocots [5]. BRs are a new group of phytohormones that perform a variety of physiological roles like growth, seed germination, rhizogenesis, senescence etc. and also confer resistance to plants against various abiotic stresses [6].

The work with dwarf and de-etiolated phenotypes and BR - deficient species of some *Arabidopsis* mutants were rescued by the application of BRs [7-8]. The present study is undertaken to understand the effect of application of 24-epibrassinolide and 28-homobrassinolide on activities of two oxidizing enzymes (catalase, peroxidase) and two hydrolyzing enzymes (ribonuclease, protease) of radish plants.

MATERIALS AND METHODS

Chemicals and plant material

The two brassinosteroids (BRs) employed in the study, viz., 28-homobrassinolide and 24-epibrassinolide were purchased from M/s. Beak Technologies Inc., Brampton, Ontario, Canada. Seeds of radish (*Raphanus sativus* L. var Pusa chetki long) were obtained from National Seeds Corporation, Hyderabad, Andhra Pradesh, India.

Pot culture

The experiments were conducted in the glass house, Dept. of Botany, Osmania University. The plants were grown in clay pots containing fresh sieved red soil mixed with farmyard manure. Six seeds of radish were sown in each pot of depth of 1.5cm. Ten days after germination, thinning was done and two healthy seeds were retained in each pot. Plants were grown in a glass house under natural day length. 24-Epibrassinolide and 28-homobrassinolide were supplied to the plants as foliar spray at three different concentration levels viz., 0.5 μM, 1.0 μM and 3.0 μM on 20th, 35th and 50th day (from the day of sowing). In addition water treated controls were maintained. The plants were regularly watered with tap water.

Priming of leaves for enzyme assay

Leaves were primed on the 55th day for enzyme studies. Leaves from the middle part of the crown were harvested in the early hours and washed with distilled water and kept in an ice box. The glass ware, pestle, mortar and all the solutions and buffers were pre-chilled in a deep freezer before use.

The two different categories of enzymes studied were:

a) Oxidizing enzymes: Catalase, peroxidase

b) Hydrolyzing enzymes: Ribonuclease and protease

Catalase and Peroxidase: The leaf material was homogenized in chilled phosphate buffer (pH = 7). The homogenate was filtered and used for assaying catalase and peroxidase.

Catalase (E. C. 1.11.1.6.)

Catalase activity was assayed by the method of Barber [9].

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The reaction mixture contained enzyme extract, hydrogen peroxide and phosphate buffer (pH = 7). The reaction was stopped by adding conc. sulphuric acid and the residual hydrogen peroxide was titrated with potassium permanganate. The activity was calculated by the following formula.

C = 25/2 x 0.0017 x v/w. Where w = fresh weight of tissue in grams, v = difference in the titre value between the blank and the sample.

Peroxidase (1.11.1.7)

Peroxidase activity was assayed by adopting the method of Kar and Mishra [10]. The assay mixture for peroxidase activity contained phosphate buffer (pH = 7), pyragallol, hydrogen peroxide and enzyme extract. After incubation, the reaction was stopped by adding conc. sulphuric acid. The amount of purpurogallin formed was estimated by measuring the absorbance at 420 nm

Ribonuclease (E.C.3.1.27.5)

The leaf material was ground with potassium acetate buffer (pH = 6.5) and centrifuged. The supernatant was used as enzyme extract. RNase activity was estimated by Corbishley *et al*. [11] method.

Protease (E.C.3.4.22.44)

200mg of the leaves was homogenized in a pre chilled mortar and pestle using 10ml of chilled 0.2M sodium acetate buffer(pH=5.2 The supernatant was used as enzyme extract and protease activity was estimated of the amount of protein present according to Lowry *et al*.[12] method.

RESULTS AND DISCUSSION

The effect of 24-epibrassinolide and 28-homobrassinolide on the activities of the oxidizing enzymes present in the radish plants were not similar (Table 1). The activity of catalase extracted from 24-epibrassinolide and 28-homobrassinolide treated radish plants was more as compared to the untreated control plants (Table 1). Catalase enzyme constitutes the major part of the antioxidative system of the plants that scavenge the ROS (reactive oxygen species) and toxic substances and also detoxify the harmful H_2O_2 formed during the metabolism, which are lethal to the plants. Fariduddin *et al*. [13] observed that 28-homobrassinolide increased the catalase activity in *Brassica junceae* exposed to different levels of copper. Similarly Mazorra *et al*. [14] also observed that BRs enhanced the catalase activity in tomato plants grown under different temperatures. The present study also revealed that exogenous application of 24-epibrassinolide and 28-homobrassinolide to radish plants increased the catalase activity.

The radish plants treated with 24-epibrassinolide and 28-homobrassinolide showed lowered contents of peroxidase enzyme (Table 1). Similar reduction of peroxidase activity in 24-epibrassinolide treated hypocotyls of light grown cucumber seedlings [15] and mung bean epicotyls [16] was observed. Anuradha and Rao [17] stated that 28-homobrassinolideand 24-epibrassinolide reduced the peroxidase activity of radish seedlings grown under cadmium stress. Vardhini and Rao [18] reported that BRs lowered the polyphenol oxidase as well as peroxidase activity in tomato plants. The results obtained in case of peroxidase activity in the present study with whole plant system are in confirmity with the earlier observation made using with epicotyls and hypocotyls. Moreover it has been reported by He *et al*. [19] that the enhancement of senescence, the growth retreating phase of growth, as induced by epibrassinolide in the leaves of mung bean seedlings was associated with enhanced peroxidase activity.

24-Epibrassinolide and 28-homobrassinolide application resulted in reduction in the activity of the enzyme ribonuclease in the radish plants compared to the control plants (Table 2). Elevated activity of RNA polymerase and lowered activity of RNase and DNase were observed in mung bean seedlings when treated with epibrassinolide [21]. Vardhini and Rao [22] observed reduced ribonuclease activity in tomato plants supplied with brassinolide which is similar to the results in the present study where supplementation of brassinolide to radish plants showed lowered ribonuclease activity compared to control plants.

The present study with whole plant system revealed reduced protease activity in 24-epibrassinolide and 28-homobrassinolide - treated radish plants (Table 2). Seed treatment of BRs to the four varieties of sorghum seedlings grown under PEG-imposed water stress exhibited reduced protease activity [23]. The supplementation of BRs to wheat plants [24] and rice seedlings [25] resulted in enhanced soluble proteins under various stress conditions. The decrease in the protease activity might have been due to reduced protein degradation and *denovo* polypeptide synthesis.

Table 1. Effect of brassinosteroids on the oxidizing enzymes of *Raphanus sativus*

Compounds	Treatments	Catalase activity^a	Peroxidase activity^b
24-Epibrassinolide	0.5µM	62.41 ± 2.76	0.676 ± 0.01
	1.0µM	73.20 ± 2.69	0.579 ± 0.04
	3.0µM	82.08 ± 2.97	0.520 ± 0.03
28-Homobrassinolide	0.5µM	65.87 ± 2.16	0.646 ± 0.02
	1.0µM	78.73 ± 2.89	0.567 ± 0.05
	3.0µM	86.79 ± 2.89	0.514 ± 0.03
	Control	42.49 ± 1.98	0.855 ± 0.05

a = Catalase activity is expressed in terms of enzyme units.
b = Peroxidase activity is expressed in terms of absorbance units which indicate the amount of purpurogallin formed.
Values are Mean ± S.E. (N=5)

Table 2. Effect of brassinosteroids on the hydrolyzing enzymes of *Raphanus sativus*.

Compounds	Treatments	Ribonuclease activity[a]	Protease activity[b]
24- Epibrassinolide	0.5µM	0.263 ± 0.01	6.13 ± 0.06
	1.0µM	0.229 ± 0.02	5.74 ± 0.03
	3.0µM	0.209 ± 0.05	5.15 ± 0.08
28-Homobrassinolide	0.5µM	0.235 ± 0.01	5.91 ± 0.03
	1.0µM	0.209 ± 0.03	5.24 ± 0.08
	3.0µM	0.186 ± 0.01	4.85 ± 0.02
	Control	0.33 ± 0.03	8.54 ± 0.06

a= RNase activity is expressed in absorbance units which indicated the amount of nucleotides formed due to depolymerization of RNA.
b=Protease activity is expressed in terms of the amount of protein destroyed in µg g^{-1}Fr. Wt. /30 minutes.
Values are Mean ± S.E. (N=5)

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